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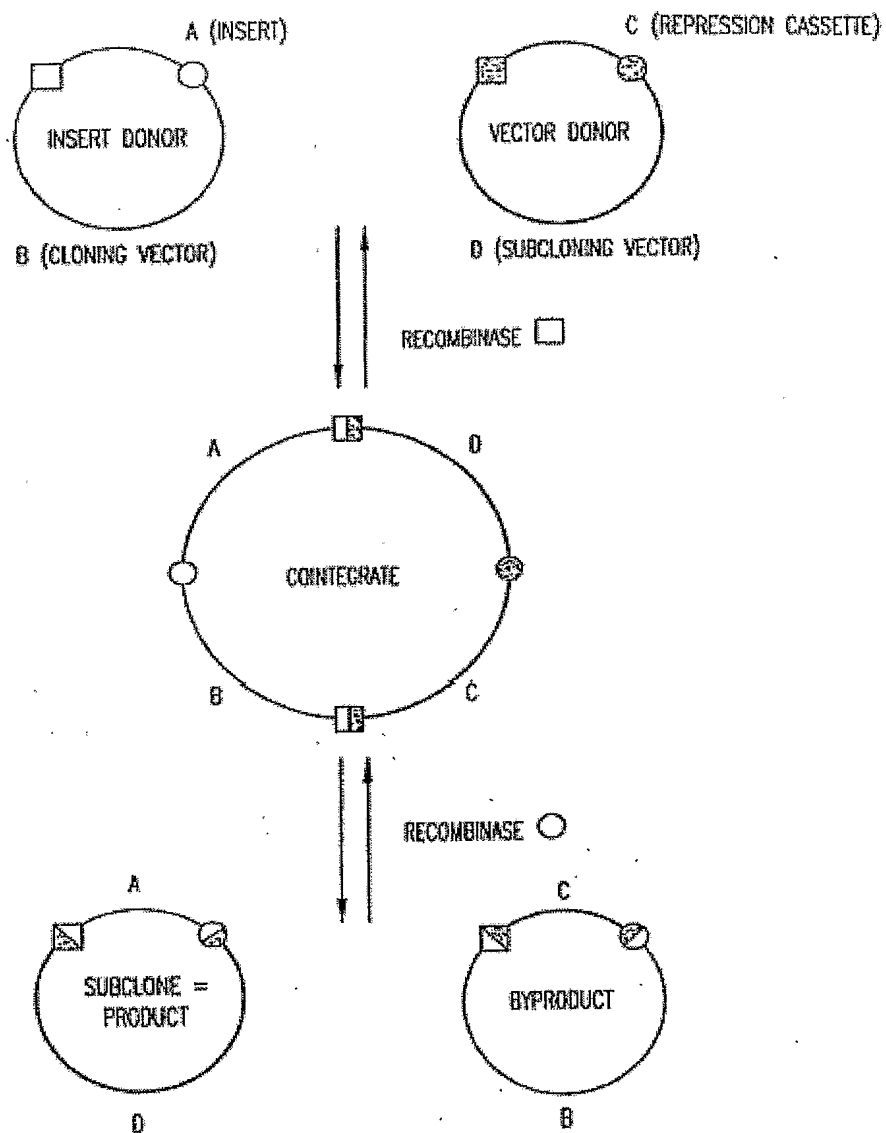


FIG.1

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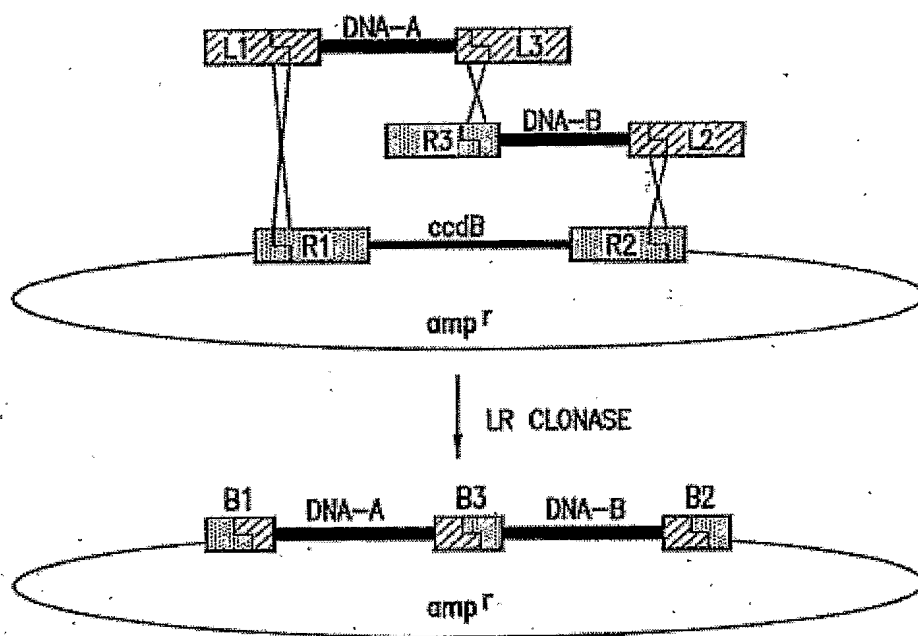


FIG.2

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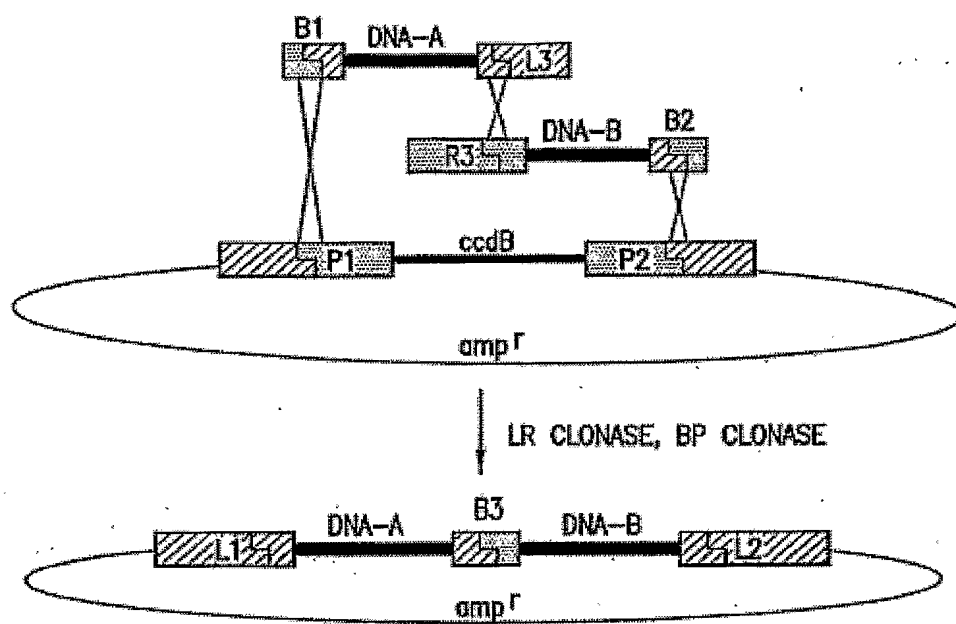


FIG.3

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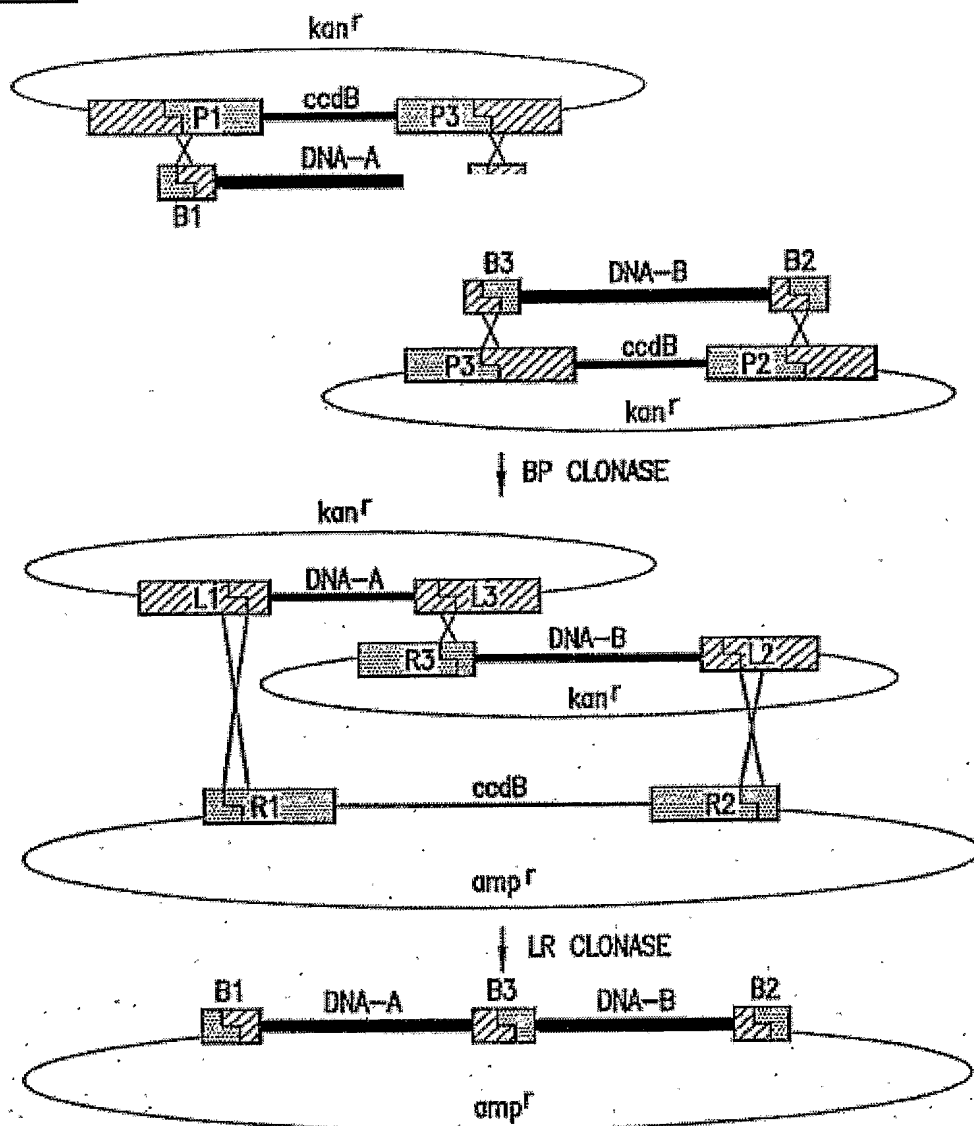
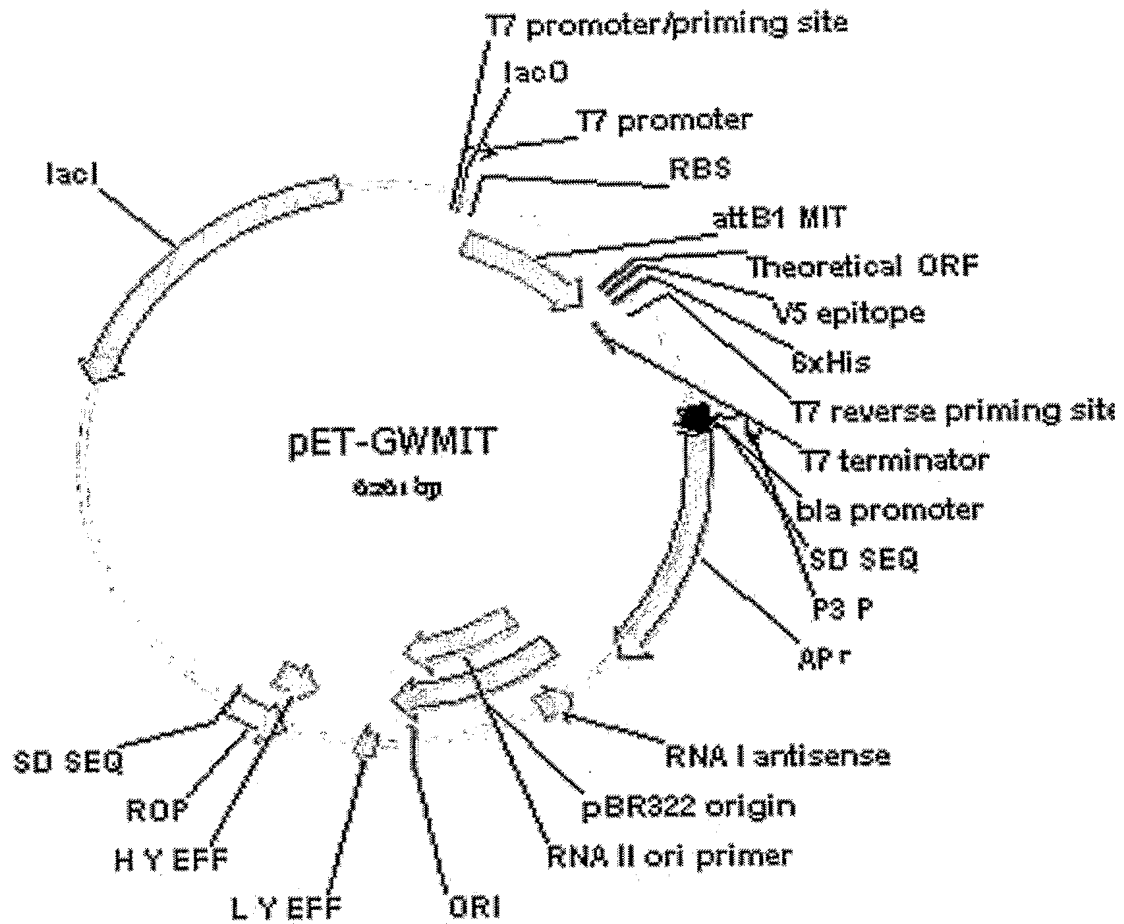
**Intein**

FIG.4

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**FIG. 5**

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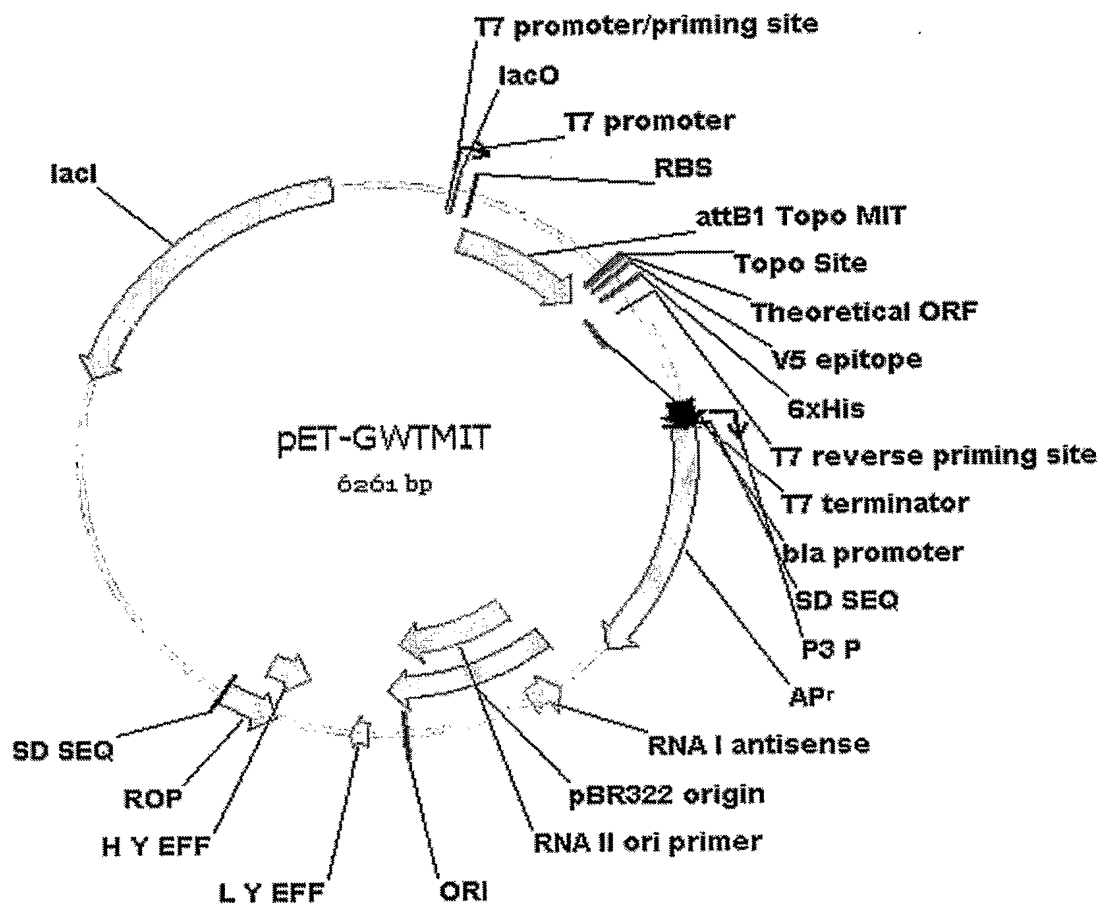


FIG. 6

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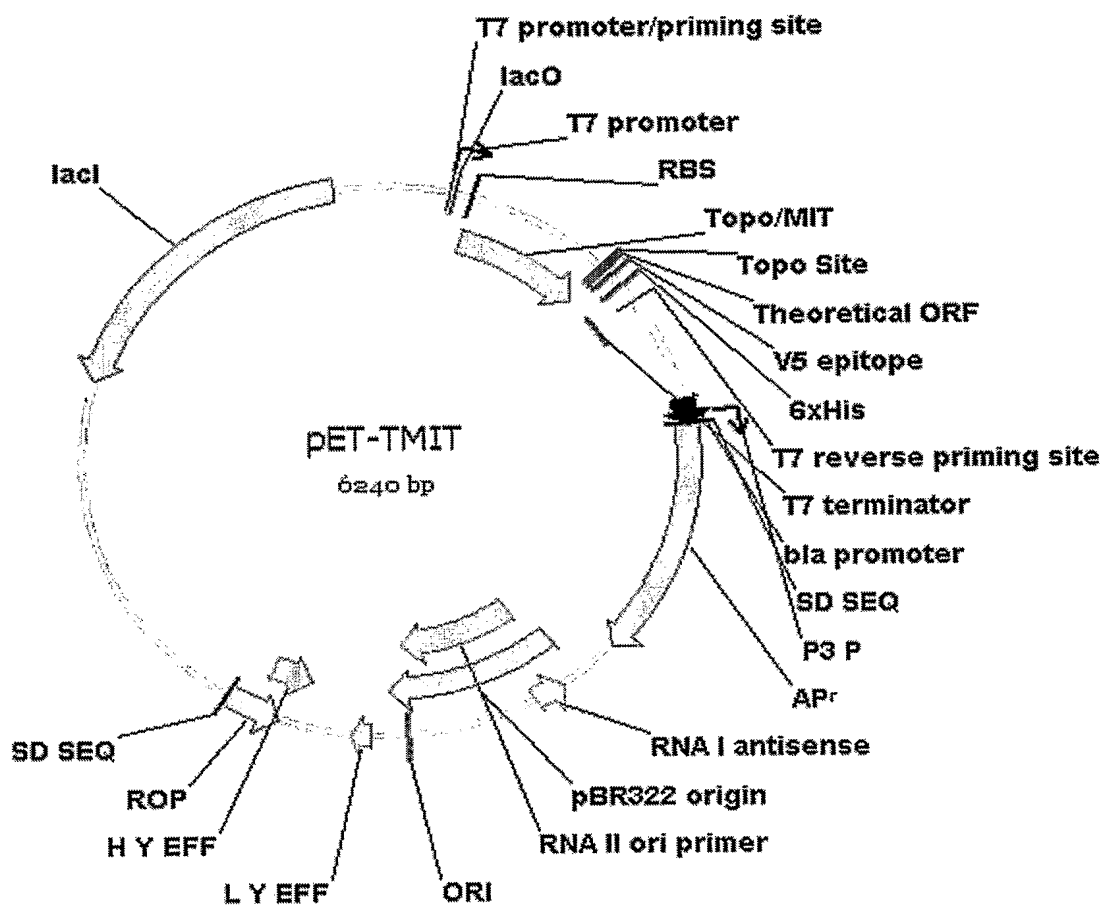


FIG. 7

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## Conserved C-terminus of the Intein

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Gene	N-Extein	Intein Sequence		C-Extein
Sce VMA	A I L Y V G	C F A K G T	-( 454 AA )-	N Q V V V H N C G E R G N
Ctr VMA	V I I Y V G	C F T K G T	-( 471 AA )-	N M A L V H N C G E R G N
Mtu recA	K V V K N K	C L A E G T	-( 440 AA )-	E G V V V H N C S P P F K
Mle recA	I G V M F G	C M N Y S T	-( 365 AA )-	D G V M V H N S P E T T T
Tli pol I2	K V L Y A D	S V S G E S	-( 390 AA )-	N N I L V H N T D G F Y A
Tli pol I1	I K L L A N	S I L P N E	-( 538 AA )-	G L L Y A H N S Y Y G Y M
Psp pol	I K I L A N	S I L P E E	-( 537 AA )-	G F L Y A H N S Y Y G Y Y

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**FIG. 8**



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## Topo Intein Designs

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Original Intein Coding Sequence	TS Coding Sequence
<div style="display: flex; align-items: center; justify-content: center;"> <div style="border-bottom: 1px solid black; width: 100px; margin-bottom: 2px;"></div> <div style="font-size: 1.2em; margin: 0 5px;">→</div> </div>	<div style="display: flex; align-items: center; justify-content: center;"> <div style="border-bottom: 1px solid black; width: 100px; margin-bottom: 2px;"></div> <div style="font-size: 1.2em; margin: 0 5px;">→</div> </div>
GTT GTT GTA CAC AAC TGT	ATG AAA CAA TAC
CAA CAA CAT GTG TTG ACA	TAC TTT GTT ATG
Val Val Val His Asn Cys	Met Lys Gln Tyr

### "ALV Intein"

Topo Mutations

<div style="display: flex; align-items: center; justify-content: center;"> <div style="border-bottom: 1px solid black; width: 100px; margin-bottom: 2px;"></div> <div style="font-size: 1.2em; margin: 0 5px;">→</div> </div>	<div style="display: flex; align-items: center; justify-content: center;"> <div style="border-bottom: 1px solid black; width: 100px; margin-bottom: 2px;"></div> <div style="font-size: 1.2em; margin: 0 5px;">→</div> </div>
GCC CTT GTA CAC AAC TGT	ATG AAA CAA TAC
CTT GAA CAT GTG TTG ACA	TAC TTT GTT ATG
Ala Leu Val His Asn Cys	Met Lys Gln Tyr

### "VLV Intein"

Topo Mutations

<div style="display: flex; align-items: center; justify-content: center;"> <div style="border-bottom: 1px solid black; width: 100px; margin-bottom: 2px;"></div> <div style="font-size: 1.2em; margin: 0 5px;">→</div> </div>	<div style="display: flex; align-items: center; justify-content: center;"> <div style="border-bottom: 1px solid black; width: 100px; margin-bottom: 2px;"></div> <div style="font-size: 1.2em; margin: 0 5px;">→</div> </div>
GTC CTT GTA CAC AAC TGT	ATG AAA CAA TAC
CAT GAA CAT GTG TTG ACA	TAC TTT GTT ATG
Val Leu Val His Asn Cys	Met Lys Gln Tyr

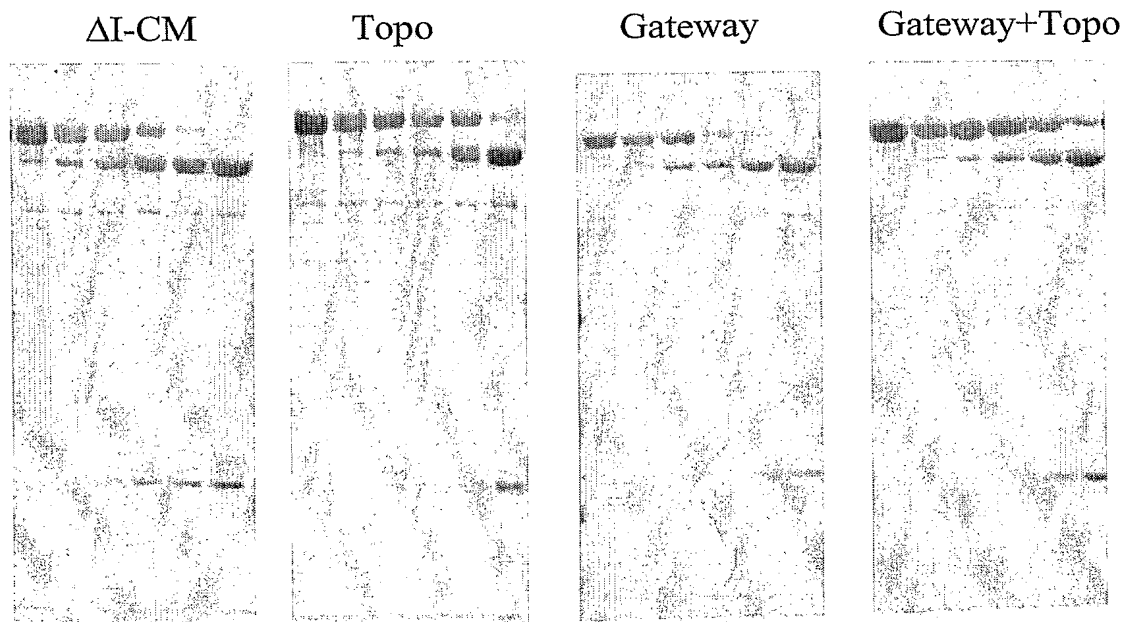
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FIG. 9

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### Cleaving Rate Studies

(Cleaving at pH 7.0 for 0, 1, 2, 4, 8 and 22 hrs)

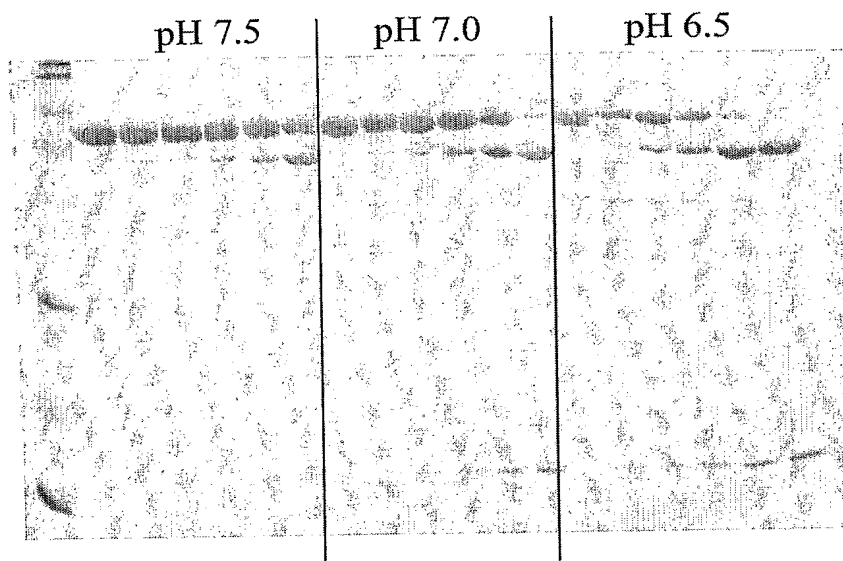


**FIG. 10**

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## **Cleaving Rate Studies - Optimal pH**

(Cleaving at 37°C)



**FIG. 11**